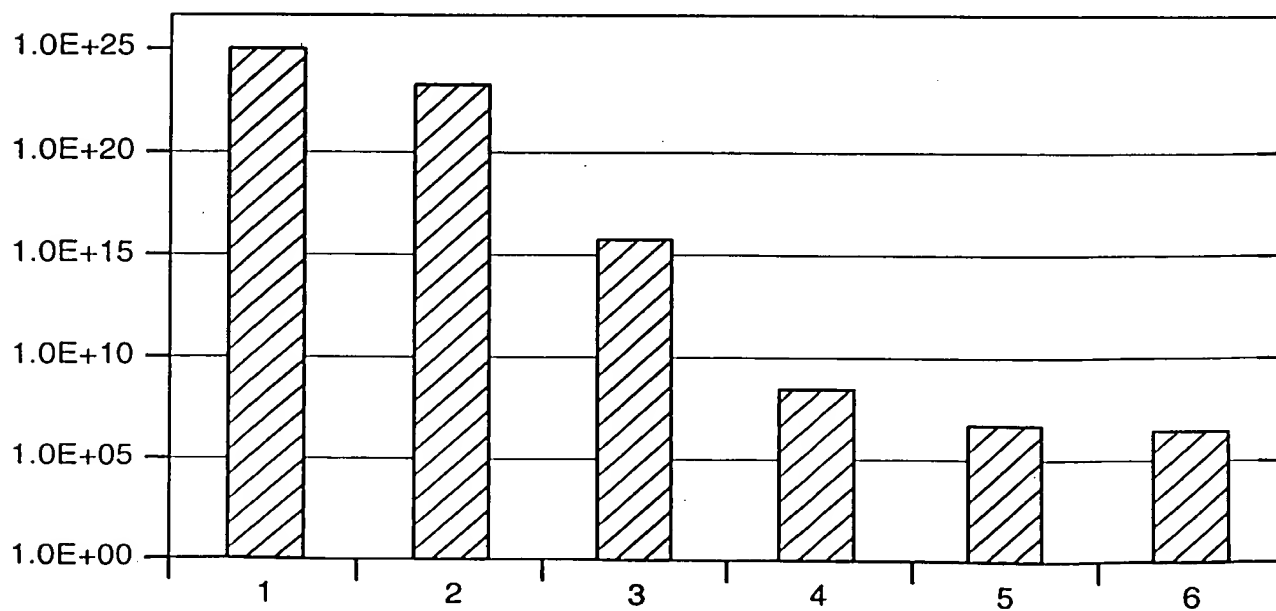


Figure 1 consists of five schematic diagrams labeled 1 through 5, illustrating different types of DNA damage. Each diagram shows a double-stranded DNA molecule with a top strand and a bottom strand. 1. A single-strand break (SSB) is shown as a gap in the top strand. 2. A double-strand break (DSB) is shown as gaps in both the top and bottom strands. 3. A base pair deletion (BD) is shown as a missing base pair in the top strand. 4. A base pair insertion (BI) is shown as an extra base pair in the top strand. 5. A base pair substitution (BPS) is shown as a base pair in the top strand that has been replaced by a different one.



09782004.021201
102120.4002860



FIG. 3

09782604 021201
102120 40028760

+

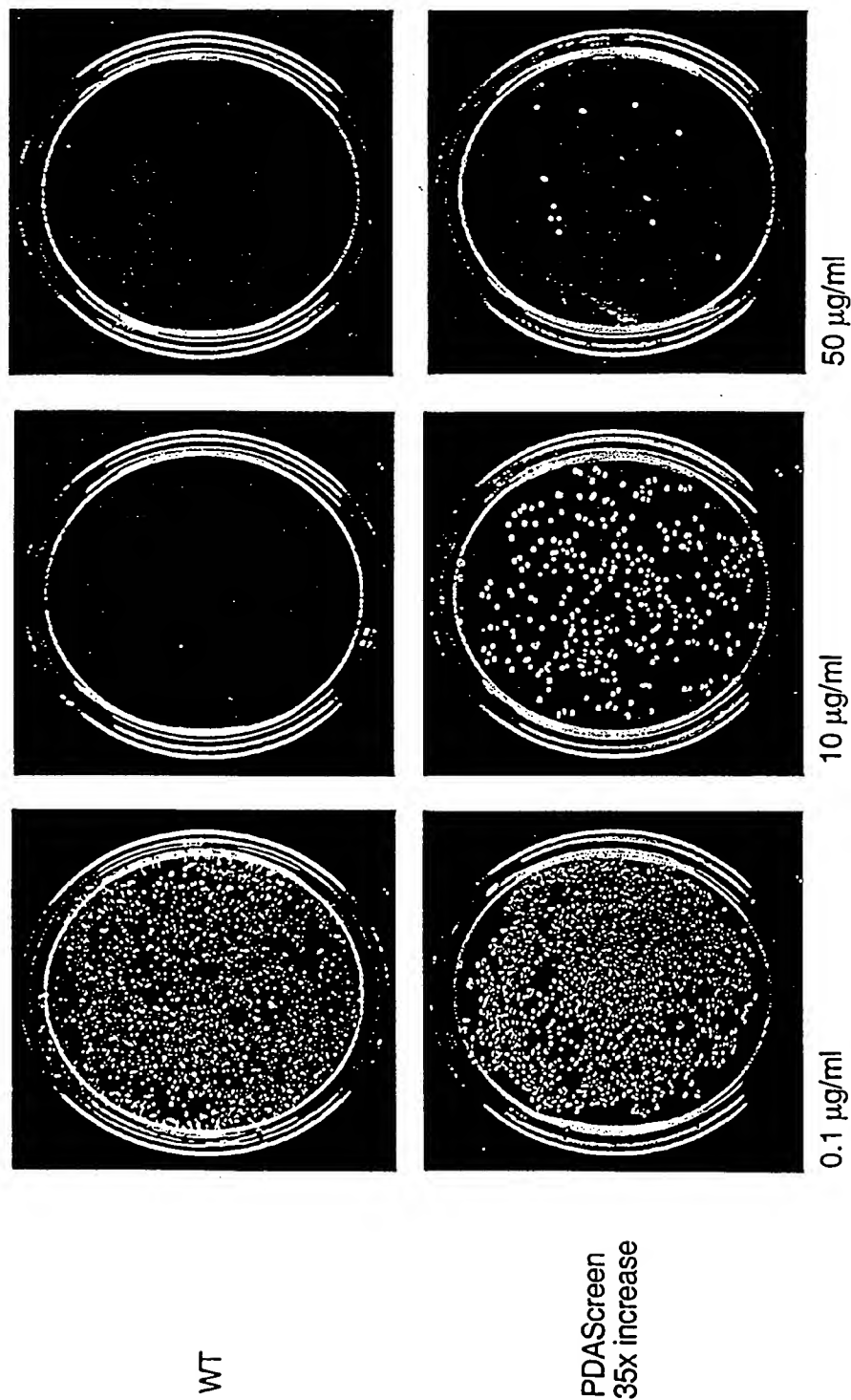
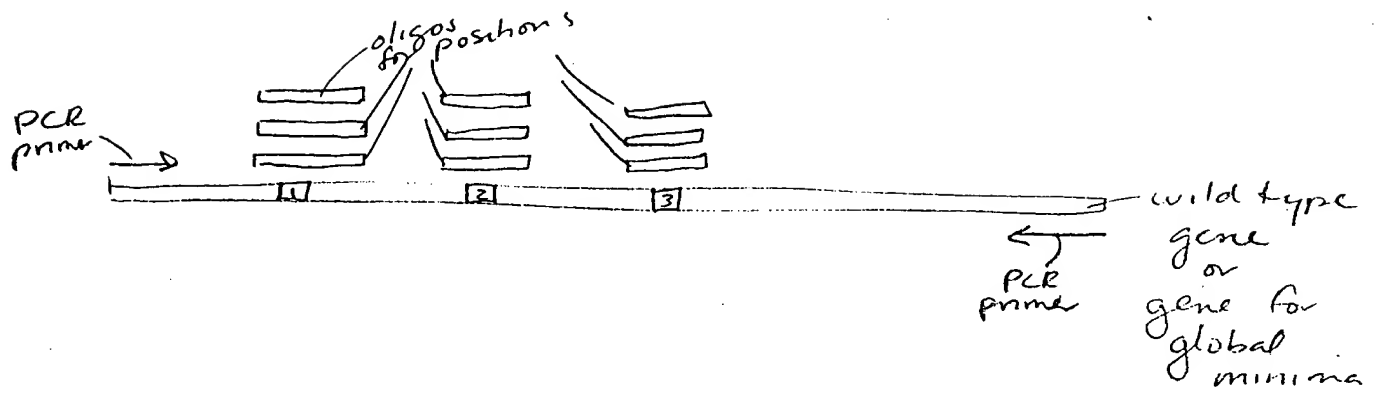


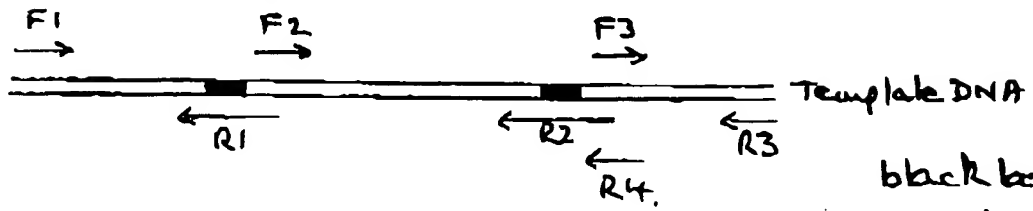
FIG. 4

Figure 5



05783004 034304
T00T00 40030250

DIAGRAM

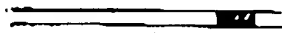


black box
= region to
be mutated.

Step 1: Set up 3 PCR reactions.

Products:

Tube 1:



Tube 2:

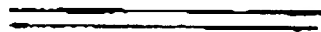
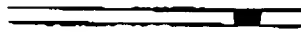


Tube 3:

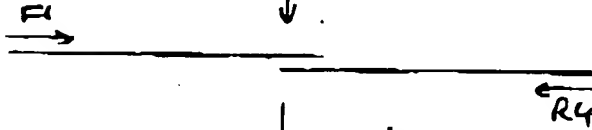


Fig 6A

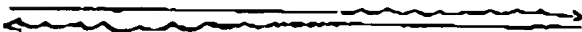
Step 2. Set up PCR reaction with products of tube 1
+ products tube 2 + F1 + R4



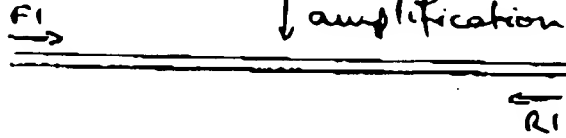
↓ Heat + anneal phase of PCR,



↓ synthesis phase of PCR.



↓ amplification phase, using F1 + R4.



during
subsequent cycles.

Step 3

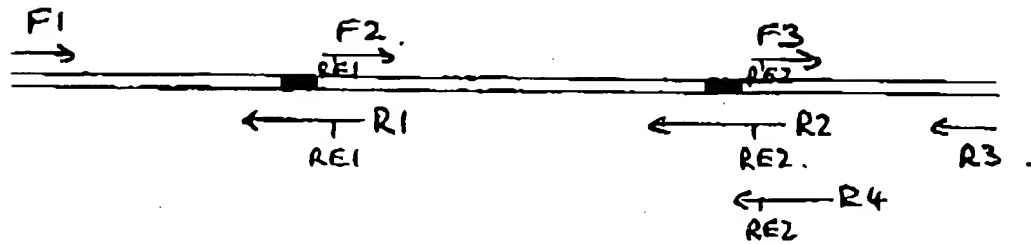
Repeat step 2 using product from step 2 +
product from Step 1, tube 3 + primers F1 or R3.

Fig 6B

09782004 091204
T02T20 40028/60

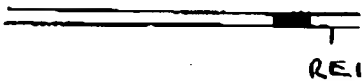
DIAGRAM 2

Fig 7A

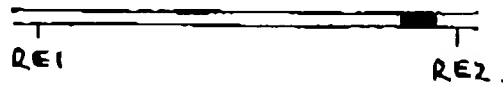


Step 1 Set up 3 PCR reactions:

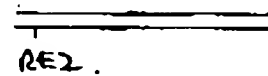
Tube 1:



Tube 2:



Tube 3:



Step 2: digest products from Step 1 with suitable restriction endonucleases

Step 3: ligate digested product from Step 2, Tube 2 with digested product from Step 2, Tube 1.



Fig 7B.

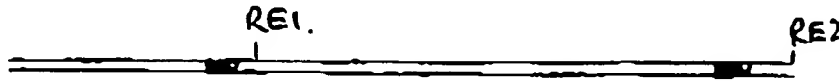
Step 4

Step 4 Fig
Amplify ^{via PCR} ligated products of Step 3 with F1 & R4.



Step 5.

Step 5. Digest amplified product of step 4 with restriction endonuclease #2.



Step 6.

h. Ligate product from step 5 with product from step 2, tube 1.

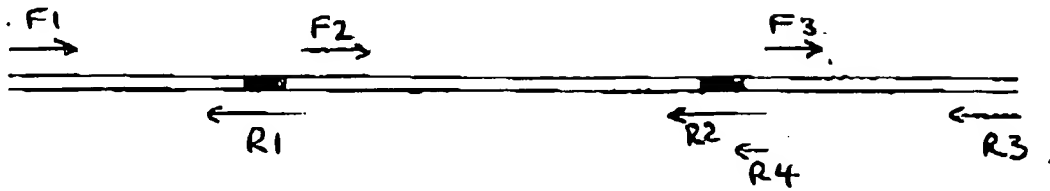


Step 7

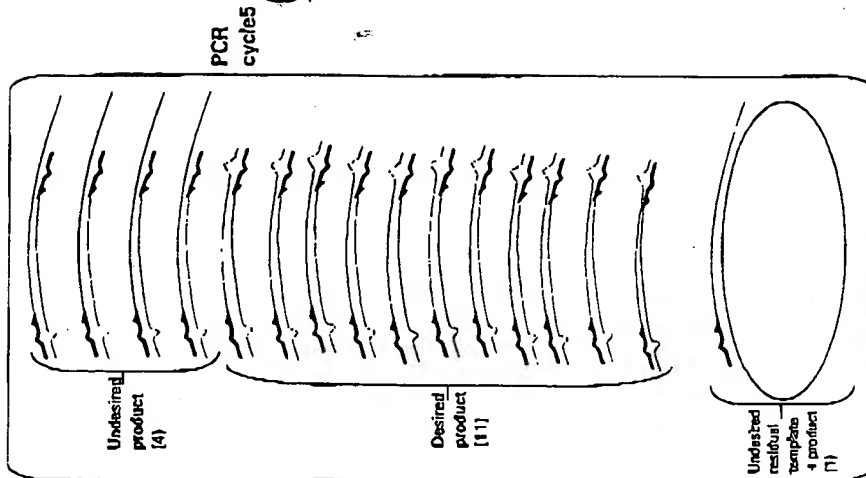
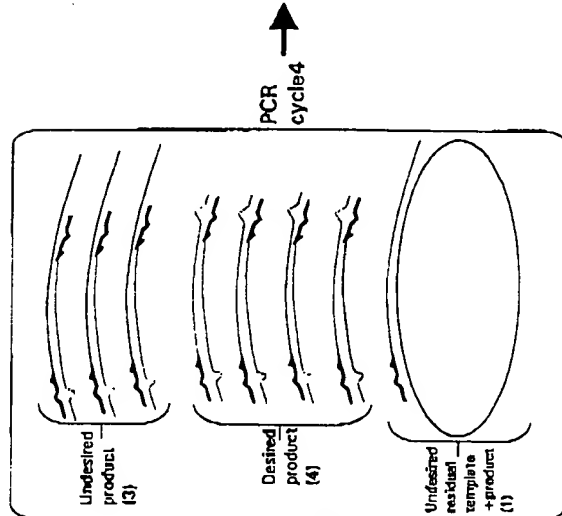
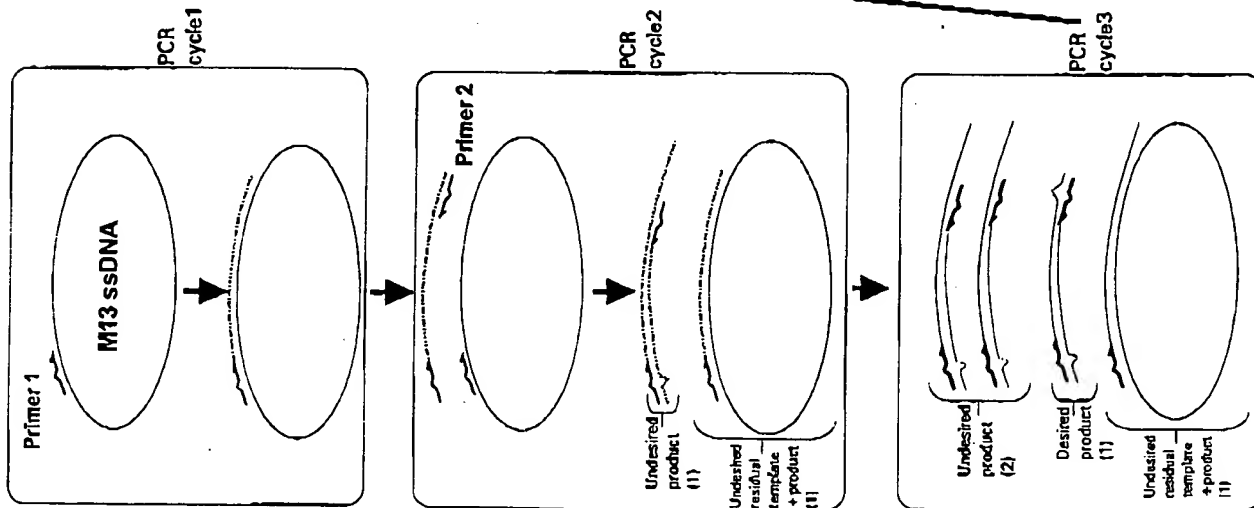
Amplify product from step 6 with
F1 + ~~B~~ R3

Diagram 3

Fig 8



Amplification Scheme Based on M13 Single Stranded Template



Numerical progression of desired product with increasing PCR cycles

PCR cycles	Desired Product	Undesired Products and residual template	Percent Desired Product in Total Product
1	1	1	0.00%
2	1	1	0.00%
3	1	1	25.00%
4	4	4	50.00%
5	11	5	68.75%
6	28	6	81.25%
7	57	7	89.06%
8	120	8	93.75%
9	247	9	96.48%
10	502	10	98.05%
11	1013	11	98.93%
12	2036	12	99.41%
13	4083	13	99.68%
14	8178	14	99.83%
15	16369	15	99.91%
16	32752	16	99.95%
17	65519	17	99.97%
18	131054	18	99.99%
19	262125	19	99.99%
20	524258	20	100.00%

Figure 9